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Models, new and old, for growth of retino-tectal connections

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In recent years some controversy has developed in the literature that deals with the growth of nerve connections between the eye and the brain. In brief, our original explanatory model first advanced in the early 1940's [12, 14] is now being questioned in the light of new and apparently conflicting findings. The resultant uncertainties in the evidence and its interpretation have prompted us to undertake some further experimental checks.

First, however, let me review a little of the background. The first Figure shows the eye and the brain of the goldfish on which much of the work has been done. This is an extremely simple system in which nerve fibers arising from the ganglion cell layer of the retina of the eye grow centrally to connect directly with the optic lobe or tectum of the midbrain on the opposite side. The basic plan of this retinotectal system is much the same in all of the lower vertebrates. The main point at issue is our conclusion from early findings in the 1940's and 50's that fibers of the optic nerve grow selectively along specific central routes to reach specific central target points in the tectum, to establish thereby an orderly topographic map of the retinal field on this brain center.

This same kind of orderly mapping is found not only in the initial development in the embryo but also in regeneration of the optic nerve in the adult when the nerve is divided and the hundreds of thousands of fibers are scrambled. The scrambled fibers somehow unsort themselves in regrowth and manage to regain their original terminations. This holds even when the eye is rotated or inverted so that the animals see everything upside down and

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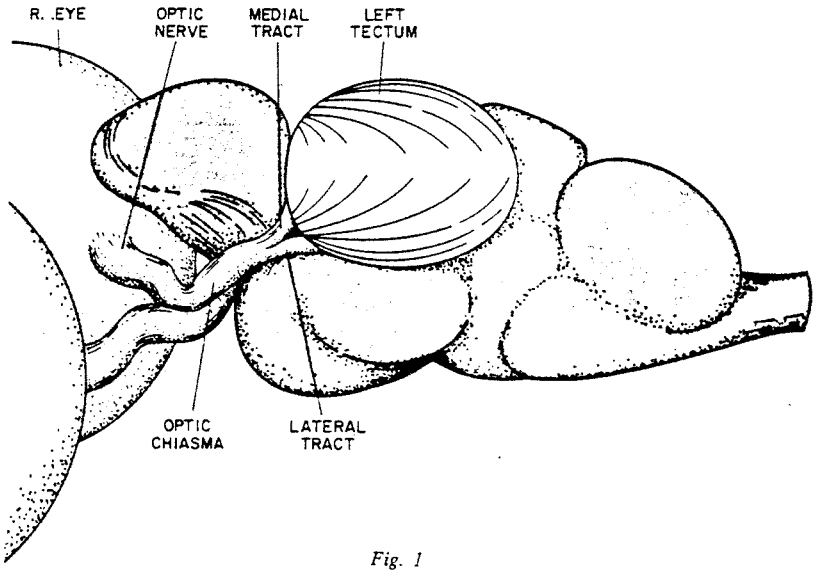

Fig. 1

backward after regeneration, and also when the nerves are crossed to the wrong side of the brain (see Figure 2) to produce a left-right reversal in visual perception. The resultant maladaptations in visual orientation remain uncorrected by experience.

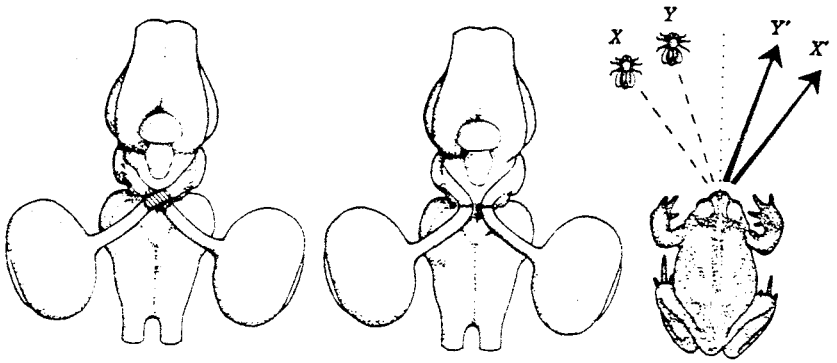

Fig. 2

We concluded from these and related findings that each retinal point is preprogrammed to connect with a corresponding complementary point in the tectum. It was inferred that the nerve cells of the retina and tectum must

acquire cell unique cytochemical tags that serve as markers to identify each cell and its fiber according to the location of the nerve cell body within the retinal or tectal field. Each locus in the retina was inferred further to have a corresponding complementary or matching locus in the tectum for which it, or it's fibers, possess a selective preferential chemical affinity.

This kind of refined chemical labeling of many thousands of individual cells in an adult tissue that seemingly is homogeneous in appearance was found a bit hard to accept back in those days, and apparently still is in some places. We suggested [12] that such chemical labeling could most easily be brought about by a polarized field-like or gradient type of differentiation on at least two, perhaps three, axes in the developing retinal and tectal fields. The latitude and longitude of each cell in the field is stamped on the cell, so to speak, giving a locus specificity expressed in chemical terms.

The same scheme with slight modification will work also for the visual pathways of higher forms including man. It has also been invoked to account for experimental findings on other systems like the vestibular and cutaneous [12, 14] where we find the same kinds of gradients and topographic mapping in the brain centers. In other words this retino-tectal model and the points that are at issue here involve general principles that have wide applicability to the formation of nerve connections throughout the brain and nervous system.

We are ready now to consider some of the more recent findings obtained largely by GAZE and his associates [3, 6]), by SHARMA [11], by YOON [15, 16], and others that have seemed to not conform with the original interpretation. Compound eyes formed experimentally in the early amphibian embryo by uniting two nasal half retinas or two temporal halves are found to develop their tectal projections not to just the corresponding half tectum as might be expected, but rather to the whole tectum, each half retina spreading across the full extent of the tectum with the two maps overlapping in a mirror image arrangement. In other experiments it is reported that when the posterior half of the tectum is removed in the goldfish the whole retina will in time come to project in an apparently orderly but compressed pattern upon the remaining half tectum. Such compression must of necessity imply a considerable rearrangement of retino-tectal connections throughout the remaining rostral half of the tectum. Similarly it has been reported that when half of the retina is removed in goldfish and sufficient time is allowed, the remaining half retina will eventually expand its half field projection to effect an orderly mapping across the whole extent of the tectum. Again this would necessitate a considerable reorganization of the original pattern of the retino-tectal synapses.

These and similar findings have been taken to mean that the old explanatory model is not adequate. A modified hypothesis is accordingly pro-

to test this recently using the adult tree frog *Hyla regilla* [9, 10]. Repeating the procedures used in some of the previous experiments, the posterior half of the tectum was ablated, the resultant blind area mapped, and then the optic nerve was divided and allowed to regenerate. If the sliding scale model is correct, one would expect optic nerve regeneration to result in a compression of the whole visual and retinal field on to the remaining front half of the tectum (see Figure 3). By contrast, according to the original model one would expect not to find such a compression and that the blind area or scotoma would remain unchanged.

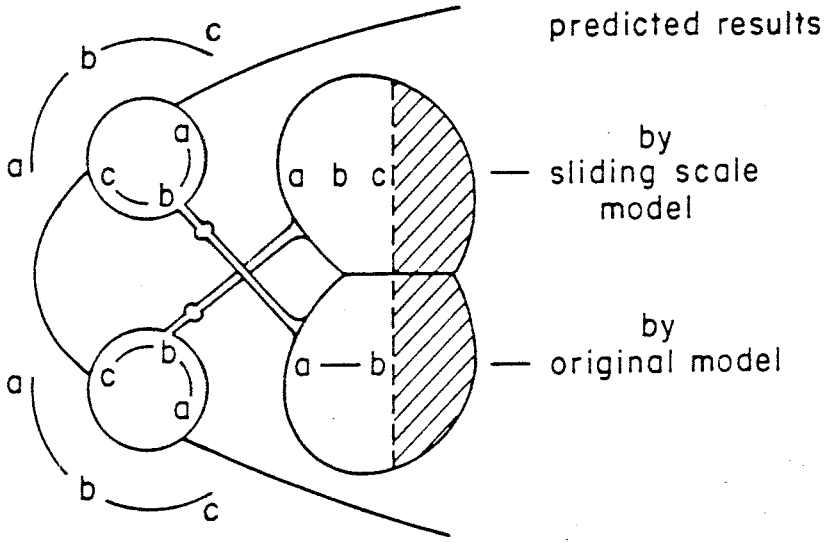

Fig. 3

The results were clear-cut throughout in favor of our original explanation [10]. The regenerating fibers, that is, reconnected with their original target loci. No evidence was obtained for compression of the retino-tectal map as reported for the goldfish, nor was there any indication of the formation of synaptic connections on a sliding scale basis. The findings were consistent in over 15 animals and the scotomata remained stabilized for periods up to 334 days after nerve section.

The results were the same whether checked by electrical mapping following the procedures of GAZE or by behavioral mapping which seems to give a somewhat more complete and more functionally reliable measurement.

Compression of the tectal map as observed in goldfish after caudal tectal

posed by GAZE in which the concept of gradients and chemical ordering is retained but in which the optic fibers, instead of finding predetermined addresses, arrange themselves instead in an orderly way to fill up whatever gradient or part thereof happens to be available. Synaptic connections are formed in this scheme on a competitive sliding scale basis, as it were, instead of by prefixed values.

Let us assume here that these findings are sound although there remains some lingering shadow of doubt about the nature and details of the adjustments reported to follow retinal and tectal lesions in the goldfish. Our position with regard to these apparent discrepancies has been that they are not necessarily in conflict and could be readily and even better accounted for in terms of the original model than in terms of the "sliding scale" concept. The polarized field-type differentiation system on which our original explanation has been based is, by definition as a morphogenetic field, something which if cut in half will automatically reorganize itself into a whole.

Thus the compound double nasal eyes, for example, by the time testing occurs, may be presumed to contain not two half retinas as assumed, but rather two whole twin retinal fields. Each half regina, backed against itself, i.e., encounters developmental pressures that force it to form a whole self-contained retinal field in terms of chemical properties, if not anatomically. This, of course, would exploin why the two original nasal or temporal halves have been found to overlap and spread each across the whole extent of the tectum with the two in mirror image alignment. Similar surgical manipulations on the developing limbbud [1] have been found to produce the growth of two separate entire limbs arranged also in mirror image position.

In regard to the reorganizations obtained after removal of the half tectum or retina and related lesions in goldfish, we likewise need merely to assume that there is still sufficient developmental plasticity so that the remaining intact portion of the tectal or retinal field reorganizes itself to form a complete field and changes accordingly the chemical labels for cell localization. Goldfish at the age employed are still growing radiply and the retino-tectal system itself is still growing by cell division.

In other words the observed plasticity and "sliding scale" effects in these experiments do not reflect, in our view, a plasticity in the process by which nerves grow and form their connections. Instead *the plasticity resides in the chemical processes by which the tectal cells differentiate and acquire their positional specificity*. The plasticity, lies largely in the organizational dynamics of the developing morphogenetic fields of the retina and tectum.

If this interpretation is correct, one would predict that these same kinds of plasticity or "sliding scale" effects might not obtain in systems where retino-tectal growth is already completed. Ronald MEYER in our laboratory undertook

lesions could also be explained in chemoaffinity terms as follows : Assume that the tectal cell specificity that controls selective synapsis is determined, not only by a balance of chemical inputs from surrounding tectal elements, as implied in gradient dynamics, but also chemical input from the terminal arbors of retinal fibers. Cell specificity at the lesion border would first be shifted to a slightly more caudal value by an increased input from target-deprived retinal fibers the synaptic zone of which normally overlaps the border cells. This caudalward shift in value would in turn allow synapsis from even more caudal fibers shifting further the border cells' specificity in a progressive process. Specificity of adjacent cells rostrally would also be similarly shifted ; and then in turn the cells adjacent to them, and so on progressively until the process stabilized.

We must take for granted that the formation of nerve connections is always a multiple factor resultant [13] in which various intrinsic growth tendencies are balanced against a series of inhibiting, stimulating and orienting conditions in the surround. The differential terminal addresses of growing fibers are "genetically" predetermined only in reference to a complex of undisturbed conditions that normally prevail.

The biochemistry behind neurospecificity remains to be worked out and offers a happy hunting ground of possibilities for molecular model builders. We outlined years ago various reasons why an explanation based simply on the diffusion through the nerve fiber tree of graded molecular densities runs into difficulties, and why we think in terms of specificity molecules that are bound to cells and incorporated within cell membranes, and why it would be convenient to have a dipole molecule or molecular complex compounded of two complementary fractions, each of which can be varied over a wide range of quantifiable steps [14]. If the complementary ratios established by the dynamics of gradient formation could then be synthesized in an immutable form and spread throughout the neurone fiber tree, this would serve to identify any number of ramifications and terminals as belonging to that one particular nerve cell and to distinguish them from fibers of all other nerve cells.

A more precise and detailed molecular model has been proposed recently by MCMAHON at our Institute, building largely on evidence obtained from differentiation of the slug of the "social amoeba" *Dictyostelium discoideum* [8]. By his model an undifferentiated array of cells could each one determine its position in the system, establish an overall gradient, and convert the positional information into a form which could be used to regulate gene expression. His model (which I do not pretend to be able to support in detail) proposes that each individual cell is polarized with different and complementary specificity molecules on the plasma membrane, front and back, dorsal and ventral, etc. The neurospecificity becomes expressed in terms of different ratios

of these complementary molecules as they are distributed in cells across the gradient. Interaction between the complementary molecules and surface membranes of adjacent cells regulates through cyclic negative feedback systems, the concentration of morphogenetic substances like cyclic AMP and cyclic GMP. The same system of molecular interactions is presumed to be involved in regulating and directing the speed and orientation of growth in the advancing nerve tip. The whole system with the complementary ratios, the negative feedback, interactions and cyclic AMP regulation, etc., is all represented, as it has always seemed that it must be [14], in the individual filopodia of the growing nerve tip.

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Discussion

EBERT : Thank you, Roger. As Aron MOSCONA observed this morning, Roger SPERRY's elegant experiments long ago were among the foundation stones which led MOSCONA and others to begin to use cell culture techniques to explore the nature of these specificities that Roger has argued for so effectively over the years. I would propose now that we have a period of general discussion, focusing on the specificity of connections in the nervous system. These questions might be directed either to MOSCONA or to SPERRY. Are there questions concerning neurogenesis for either of these speakers ?

BENZER : Has everyone understood the experiment which disproved the sliding model ? I think that Dr. SPERRY should have more time to explain this.

EBERT : Roger, would you like to diagram these experiments on the board ?

SPERRY : It seems simple enough. We repeat the earlier experiment of removing the posterior half of the tectum with and without section of the optic nerve. According to the previous results and the sliding or systems model, the retina after regeneration should project its full extent in an orderly way upon the remaining half tectum. In repeating this, however, we use an adult system where the remaining half tectal field is already maturely specified and no longer possesses its embryonic plasticity and ability to reorganize into a whole tectum. The results are then quite different in that the remaining half tectum becomes innervated, not by the whole retina, but only by the appropriate hemiretina, as predicted in our original model. In other words you don't get the sliding scale synapsis As I say, this same kind of result is seen in the chick embryo. Embryologists have long known that chicks, as compared to fish and amphibia, develop and differentiate more rapidly, i.e. their cells and tissues become specified at an earlier stage and lack the labile reorganizational capacities of fish and amphibians.

LOEWENSTEIN : I would like to address myself briefly to the question of how the cues may be conveyed, which specify the retino-tectal system, in particular, of how the connections there may be irreversibly specified. Along the way, I should like to mention a few experiments which Marcus JACOBSON and I have been doing, in an extension of your classical work. JACOBSON had shown that the cues for the connectivity of the outgrowing retinal ganglion cells can be obtained not only in the orbit, but also in other parts of the body, for instance, in the flank of the animal, if the eye is transplanted there. Furthermore, the connections are irreversibly specified within critical periods of a few hours, at — I believe — the developmental stage 30 in the embryo of *Xenopus* on which JACOBSON has been working. After the critical time, the connectivity is irreversibly determined.

Now, I had long been playing with the idea that the information for cell differentiation is disseminated via the permeable junctions throughout the embryo. In fact, as I discussed yesterday, the entire cell mass in the early embryo is interconnected by permeable junctions ; so it is quite possible that the cell-to-cell continuity required for the so called morphogenetic field in the embryo is given by the junctions.

Now then, if the junctions were indeed involved in the dissemination of morphogenetic information, such as in the specification of neuronal connection, then a simple mechanism suggests itself for the irreversibly specification of nerve connection : the cell disconnect themselves at their junctions from the general morphogenetic field. Such uncoupling is in fact known to occur under various experimental conditions in which the junctional permeability falls by an elevation of intracellular Ca^{++} concentration. One of my earlier models (LOEWENSTEIN, 1968) considers just such an uncoupling as a general possible mechanism for irreversible cell differentiation.

With this consideration in mind, Marcus JACOBSON, Birgit ROSE and I examined the junctional coupling between the retina ganglion cells in *Xenopus* embryo at the critical time of their irreversibly specification. We got some encouraging results — or I should better say tantalizing results. Many of the cells formerly coupled to each other and to the rest of the cell mass of the embryo, were found to be uncoupled at the critical time. Unfortunately, however, for a number of technical reasons for which I have no time to go into, we were unable to prove the uncoupling rigorously (Unlike coupling which is easily demonstrated, it is difficult to demonstrate uncoupling. Although we have developed procedures for the demonstration of uncoupling in cell systems that can be cultured (AZARNIA and LOEWENSTEIN, 1971), these procedures could not be used in *Xenopus* embryo). Thus the results are tantalizing ; and, at this time, at best I can say that junctional uncoupling is *a priori* a simple possibility for irreversible specification of retino-tectal connection.

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normal cells. *J. Membrane Biol.*, **6**, 368-385.

SPERRY : Yes, but we've been inclined to think of this specification process a little differently : we see the embryo as being polarized like a large morphogenetic field in itself... The antero-posterior, dorso-ventral, and medio-lateral gradients are already present in the embryo and the eye, limb, and other subfields simply emerge out of the main axial gradients. In retinal polarization we have the same basic embryonic axial gradients that are there from the beginning.

The Jacobson concept of a critical period for acquisition of specificity we interpret in somewhat different terms. We see the specificity as being there from the start, refining and becoming more highly evolved. The critical period is interpreted to be that period during which the prevailing specificities lose their reversibility. Up until this critical period they are plastic and reversible. I think that JACOBSON and certainly his younger associate, HUNT, are beginning now to think along the same lines. This critical period then is not the period for specifying or for setting in the specificities, but the period during which the cell specificities are becoming irreversibly fixed. This again is a complicated process because the cells lose the ability to reverse field polarity before they lose the ability to merely stretch or compress the field. These are two different properties of the gradient and they become determined at different times.

LOEWENSTEIN : Yes. Perhaps, I was not clear enough in making my point and your terminology is more subtle than mine. I think, if I simply don't call this specification, but

irreversible determination, there would be no disagreement. In otherwords — just to repeat — the hypothesis I have presented is that irreversible determination of neural connection results from uncoupling of the ganglion cell junction from the general morphogenetic field ; this is so regardless of where within the field the ganglion cells were before the uncoupling, whether they were in the orbit or in the flank of the animal.

SPERRY : I think STONE showed this also back in the '50's, didn't he ? — That eyes transplanted to the flank and then back to the orbit showed reversed functional polarity induced by the gradient properties of the flank ?

EBERT : Does everyone understand why LOEWENSTEIN and SPERRY agree, finally ?

SZENTÁGOTHAI : May I, perhaps, introduce here another model from the development of neural tissues that might show the mechanisms at work, when synaptic connectivity is established. We took advantage of the known fact that the parallel fibers in the cerebellar cortex grow out rather late in most mammals : in the kitten during the first to the fifth postnatal week.

The limbs of 4-5 days old kittens have been immobilized by placing them into casts (or boots) of wax, from the shoulder and pelvic girdle down to the soles. Additionally weights of lead were fixed to the distal parts of the casts. These casts were sufficiently plastic to permit the growth of the limbs, however, virtually prohibited all movements. Due to obvious anatomical reasons immobilization was much more effective in the lower limb, while movements of the shoulder could not be prohibited completely. Such kittens could survive without a major lag in growth (body weight) until the fifth week of life. When the casts were removed at this age the kittens were heavily ataxic, although they could stand up with rigidly extended limbs. The joints did not become ankylotic and the limb muscles were only moderately atrophic. A thorough electron microscopic study of the cerebellar cortex revealed quite spectacular changes in the main projection areas from the hindlimbs : particularly in lobus III (LARSELL) of the intermediate lobe. Changes in the forelimb projection area were much less pronounced, although in the same direction. From subjective inspection of the electron micrographs the impression was gained that parallel fibers contributed to a smaller fraction of the section surface area, as compared to the normal litter mates, and that synaptic thickenings of the parallel fibers were much more frequent (were found to be more densely distributed) than in the normal. A thorough stereological analysis of the hindlimb projection area showed that the ratio of granule to Purkinje cells (1790 : 1) and also the number of dendritic spines per Purkinje cell ($\pm 9 \times 10^4$) was the same as found in the adult (PALKOVITS *et al.*, *Brain Res*, 45, 15-29, 1972). Since each granule cell gives rise to one axon branching in T-shape fashion into a parallel fiber, one had to conclude that the number of parallel fibers was not changed in these experiments. The fundamental change was in the surface fraction occupied on electron micrographs by the section surfaces of parallel fibers, which was radically decreased, and in the ratio between section profiles of synaptic thickenings : thin parts of parallel fibers, which was radically increased. The only conclusion that can be drawn from this finding is that the average parallel fiber has to be much shorter than in the normal (the reduction being close to 50 %). Since the total number of Purkinje cell spines in any given volume of molecular layer was unchanged, the smaller total " parallel fiber length " had to accomodate the same number of spines, hence synapses between Purkinje dendrite spines and parallel fibers had to be more densely distributed than normally. Normally (PALKOVITS *et al.*, *Brain Rese.*, **34**, 1-18, 1971) each parallel fiber appears to establish a synapse only with one from five (or six) Purkinje cells whose dendritic tree it crosses. In the immobilized kitten this contact ratio appears to rise to one synapse esta-

blished with every third or even with every second crossed Purkinje cell. The cerebellum may be, of course, a very special case, where — as known since several years — the Purkinje cells grow out spines irrespective whether there are presynaptic elements to contact them. But even so this model would illustrate a mechanism of synapse and connectivity development governed entirely by " supply " and " demand ", *i.e.* so many Purkinje spines are present in any volume of molecular layer and they are supplied with synapses by whatever parallel fibers are available in the neighbourhood : if the parallel fibers are of normal number and normal length (2 mm) the outcome is that each parallel fiber gets one synapse for every five (or six) Purkinje dendritic trees crossed, if the parallel fibers do not grow out to normal length — as it seems to occur in consequence of gross impairment of function — they can (or have to) make more synapses accordingly because relatively more spines are available. — Such models cannot be generalized, however, it is quite general in the so called " microneurons " of the CNS to develop relatively late in histogenesis. Particularly their axons grow out to their final richness and span of the arborisation only in the postnatal period, hence normal function during a critical period of growth might well be an essential link in the establishment of normal or — if such function is lacking — subnormal synaptic connectivity.

LOEWENSTEIN : What does the zone of contact look like, between that cell which wraps itself around and the other cell ? Does one recognize any type of structure in the electronmicroscope of the kind we know now to be associated with the channelled form of communication I talked about yesterday ?

EBERT : I think LOEWENSTEIN's question shows how one's biases come through. LOEWENSTEIN looked at this photograph and obviously saw his " channels " and LEVINTHAL saw GEREN's demonstration of the inrapping of the myelin sheath around the axon. I think these examples have focused our attention on the subtely and the unique specificity that has to be established in the developing organism. I think we have spent enough time on the complexities and we ought now to discuss MOSCONA's dissection of these questions at the cellular and molecular levels.

MOSCONA : To make sure that we don't get into misunderstanding about terminology : Concanavalin A *agglutinates* cells : it immobilizes cells and thereby prevents morphogenetic processes ; it is, of course, a very useful experimental chemical probe for membrane studies. However, I was referring this morning to *cell aggregation* in the *morphogenetic* sense, in other words, to specific constituents which mediate cell contacts conducive to tissue formation and organization. I think this distinction is worth pointing out to prevent future misunderstandings due to using the same term (agglutination) for different phenomena. As to your theoretical points, I fully agree with the idea that the attachment of ligands to the cell surface might change the configuration of the cell surface, resulting in flow within the cell membrane.

EBERT : Are there other questions directed specifically to the nature of ligands as MOSCONA has discussed them, or the nature of cell receptor sites ? If not, I think we ought to focus our attenion inward, that is, how do changes at the cell surface influence the inner workings of the cell ? There are a number of phenomena in which cell surface changes have a direct effect on the cell nucleus. DULBECCO, would you start the discussion ?

DULBECCO : What I want to discuss briefly are certain contact phenomena which can be observed in tissue culture. They are probably quite different from the phenomena that occur in differentiation, they may be simpler. But I think they do reveal a potential

response of the cells, which almost inevitably must have some relevance on more complex phenomena. In cultures, the fiblastic cells which Dr. SACHS talked about are egnerally grown on a surface of glass or plastic on which they have a few anchoring points. The cell is surrounded by medium, above it, below it on every side ; when they tend to move, they display the movement of a forward edge in that direction. Finally the edge will find another point of attachment and the whole cell will follow behind. Several years ago ABERCROMBIE has shown that in a culture in which these cells are moving around, if two such cells meet each other, the cell that hit the other one stops. This phenomenon has been described as contact inhibition of movement. In fact what happens is that the lamella, which is the forward part of the moving cell, will stick somewhere on to the other cell and this seems to cause the cessation of the whole movement process. But then in time one notices that a new lamella will now form in the opposite direction so that the cell will now move backwards after a while and in effect the meeting of two cells is some kind of a collision because the new direction of the movement is very much at random. One impression obtained from this observation is that there is considerable randomness in the way cells behave. If one takes now a dense culture and removes a strip of cells, generating a wound surrounded by cells, at first sight it seems that the cells migrate in a kind of directional way toward the center of the wound, but if one examines the process by time lapse photography one notices that is not the case, because at any time direction at which individual cells are moving is entirely random — they're going in every direction. But they collide. And the collision forces them to back away from each other and therefore the cell migration, which might seem to be well oriented, in effect is a true diffusion. So the overall orientation is produced by the collisions.

These phenomena are very clear. I don't think there is much doubt about them. A somewhat more doubtful phenomenon which some people have recogned to be connect to contact inhibition, is that if a culture of these cells is allowed to grow in a standard medium until the cells touch each other, the culture stops growing. Somehow the notion has developed that this is another consequence of contact. It turns out that the phenomenon is much more complicated than that, because the cells require specific factors in the medium and when the cultures become confluent, the specific factors also become exhausted. In effect, it has always been extremely difficult to devise an experimental procedure by which one can differentiate between these two things effects of contacts and serum exhaustion. I mentioned last time, if a culture of this type is wounded and one looks at the cell that migrate into the wound, one finds that they are in a growth state. This has been attributed to the stretching phenomenon that I described last time, and perhaps also to the greater availability of nutrients in these conditions.

A simple experiment would probably convince you that actually for fibroblastic cells, the limitation of factor in the medium is the maior event that limits growth. The experiments consists of placing a certain number of cells in the same volume of medium and factor, but in containers of different sizes, so that everything is identical except the distance between he initial cells. With the fibroblastic cells, the number of cells obtained at saturation is the same in the various dishes which means that the distance between cells doesn't play a role. However, for certain other cells, such as epithelial cells, which tend more to stay with each — the type that MOSCONA described this morning — the factor requirement is minimal whereas the size of the container is important. Furthermore if fibroblastic cells, which require the serum factor, are given the factor in an unlimited amount, they also will finally reach a point where the size of the container is somewhat limiting. This shows that there is something inhibiting in the crowding, the reciprocal elbow room between the cells, but is it contact ? I don't know. It could be a requirement for a minimal attachment size.

The last point that I'd like to make refers to what Dr. MOSCONA described this morning, concerning the differentiation in aggregates of retinal cells. Why is aggregation important ? On the basis of the experiments that I reported, one can see two different possibilities. One is that aggregation is important because the cells touch each other. The other alternative is that after the cells aggregate, they become limiting for something that is in the medium. If so differentiation would not be the consequence of aggregation, but starvation for some factor. In effect we know that in tissue cultures, when they reach the stage in which they don't grow any more, because they have exhausted factors in the medium, they undergo some kind of differentiation and develop characteristics that are absent in the growing culture.

SAFETY FACTORS AND REPAIR TURNOVER IN Na-K TRANSPORT SITES OF HeLa CELL MEMBRANES *

It is well know that the maintenance of the alkali cation (Na + K) composition of most cells by a coupled Na-K transport system at the surface underlies the maintenance of many cell functions, including volume and growth regulation, K-dependent steps in in macromolecular syntheses and other enzymatic processes, morphology, excitability, and the transports of a number of small organic molecules which are coupled to electrolyte metabolism. While ion transport is critical in regulating these cellular activities there are in turn, at a higher level of regulation, factors which control the transport. It is a truism that transport against electrochemical gradients balances leaks along these gradients, but there is a corollary that any agents which increase leaks or inhibit transport pose a threat to the cell. The questions with which we have recently been concerned relate to how the cell responds to such threats. Turnover of electrolytes is much faster than the cell generation time, i.e., the time required for the net synthesis of a whole new membrane. Other safety factors and repair mechanisms operate, and it is these we have been examining in HeLa cells. We selected transformed human cells for such studies for reasons of simplicity ; transformed cells in culture appear to be electrically uncoupled from their fellows, as Dr. LOEWENSTEIN has pointed out elsewhere in this discussion, and their transport properties are independent of cell density. In this case there is virtually no cell-cell communication, but substantial intra-cellular communication between the membrane and the interior. Like most mammalian cells, our strain of HeLa cells (S3, originally isolated by T. T. PUCK but many generations removed from his original isolate) has a high concentration of intercellular K (160 meq/L) and low Na (~ 40 meq/L) while growing in a medium of 155 Na meq/L and only 6 K meq/L. We assay transport in these cells by their handling of ^{86}Rb, which is a more convenient isotope than ^{42}K. Rb and K are competitive inhibitors of each other in the transport system. The cells transport Rb inward slightly more efficiently than they transport K, and both ions subsequently leak from the cells at rates which are indistinguishable from each other. With reference to Dr. ORGEL's remarks earlier in this meeting, it is interesting to note that Rb is a good analog and tracer for K in more activities than just transport. HeLa cells in fact grow well, synthesizing macromolecules at nearly normal rates, in a medium in which all of the K has been replaced by Rb. Ordinarily, however, we use ^{86}Rb only as a short-term tracer for the transport system.

* Research jointly supported by the U.S. National Cancer Institute and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

Labeled HeLa cells in a steady-state with respect to their ionic composition lose their label with a rate constant of 1 hr^{-1}, while the cell-cycle time for this population is about 25 hours : in other words, the principal electrolytes turn over about 25 times in each cell generation. Turnover in transport is therefore fast enough with respect to the accumulation associated with growth so that we may make steady assumptions in short experiments with no great error.

The cardioactive steroid ouabain is a useful reagent for probing this system. Ouabain binds tightly (but not irreversibly) to Na-K transport enzymes and inactivates them. Under appropriate conditions we may use radioactive ouabain to count the number of specific ouabain-binding sites on the cell surface : in HeLa cells there are 1.6-1.8 $\times$ 10^6 such sites per cell, and if all of them together are concerned with transporting K inward at a rate equal to the Rb leak, then 40 ions are being transported per second at each site. This is a minimum estimate : the true number is higher if any of the bound steroid is associated with non-transporting sites.

We may also use ouabain as an inhibitor to " wound " the transport system, but not kill it ; we can then follow recovery. If we block about two-thirds of the sites as determined by either numbers of ouabain molecules bound or by the fraction of transport inhibited and maintain the blockade of these sites, we find that in 3-5 hours (about 1/6 of a 25 hour cycle) transport has returned to normal and a whole new set of binding sites, equal in number to the number originally present, has appeared on the cell surface. The cell has repaired itself. It is important to note that the cell cannot do this repair if *all* for the binding sites are blocked. In this case the transport system is killed, there is no observable recovery, and the cell itself dies in a day or two.

The rate of repair, as measured by functional criteria, corresponds to the rate of turnover of total HeLa cell-membrane proteins as measured by radiochemical methods. For these experiments, William PROCTOR in may laboratory adopted a double-label technique from R.T. SCHIMKE of Stanford. Cells are pulse-labeled with a mixture of ^3H-leucine and ^{14}C-leucine and their membranes are isolated for analysis. As expected, the isotopes are incorporated in the same ratio as they were given to the cells. If we space in time the pulse labels of the two isotopes, their ratios in membrane proteins change to a degree which depends on the order in which they are given, the length of the spacing, and the rate of turnover of membrane proteins. The time for turnover calculated from such experiments is again 3-5 hours, corresponding to the functional observations. It is of course not certain that all membrane proteins are turning over at the same rate : in fact, we expect that they are not, although our data are not yet complete on this point.

With the use of inhibitors like cycloheximide, it is easy to show that the functional repair of ouabain-inhibited transport activity is dependent on protein synthesis. The repair is slowed, but not stopped, by compounds like colchicine, vinblastine, and cytochalasin B, none of which affect protein synthesis. This result suggests, but by no means proves, that the restoration of a normal membrane requires turnover processes including exocytosis and endocytosis.

The ouabain effect can in some respects be mimicked by growing cells in a medium very low in K, i.e. 0.2 meq/L. The cells lose intracellular K as transport is depressed because of the deficient substrate. The transport capacity however increases dramatically. If the cells are returned to a normal environment after 2 hours in the deficient medium, they are capable of transporting at more than twice the rate of control cells. At first glance it appears that the increased transport capacity in the face of decreasing intracellular K may be a response akin to an induction phenomenon ; analogously, sugar-starved mammalian cells develop a very greatly enhanced glucose-transport

capacity. In the case of electrolyte transport, however, the enhancement in low-K medium is not accompanied by an increased number of ouabain-binding sites. Transport at each site now exceeds 90 ions per second. Furthermore, the enhancement is not inhibited by cycloheximide. This whole effect may also be seen without any drugs at all by incubating the cells, in normal medium, in an ice bath for an hour. Protein synthesis stops, transport stops ,and the cells lose more than half their K. When they are re-warmed their transport capacity is again increased by more than 100 % over the controls. What we are seeing here is quite different from the first recovery experiment in ouabain (where cell K loss is in fact not very great) and reflects the nature of the transport enzyme. K-inward transport is coupled to Na-outward transport, and although in most cells in a normal medium the system is saturated with respect to extracellular K, the intracellular Na is usually well below saturating levels. When transport is inhibited either by K depletion of the medium or by cold, Na accumulates in the cells, and when conditions are returned to normal the high intracellular Na accelerates the coupled transport until the original steady-state is achieved. Although it is technically difficult to measure in these complex cells the true cytosol concentration of Na or the Michaelis constant of the transport enzyme for Na under prevailing *in vivo* conditions, these experiments do tell us that the former is less than the latter, and that the transport system functions with a safety factor greater than 2. In other words, transport can be more than doubled without the synthesis (or insertion into the membrane) of any new sites.

The following picture emerges for the regulation of electrolyte transport. The cell surface is equipped with more than a million sites (the figure $1.6\text{-}1.8 \times 10^6$ is a cell cycle average) each functioning at less than half capacity. Relying on extracellular mechanisms to maintain an appropriate milieu, these sites are relatively insensitive to alterations in the K of the environment but are sensitive to internal changes in Na. If the surface is perturbed so that ions leak more readily than usual, or some of the transport sites are inactivated, the resultant inevitable increase in intracellular Na will stimulate the still functional sites to greater than normal activity. This " wounded " level of activity need not be long maintained : within a reasonably small fraction of a cell cycle time the membrane is replaced and normal physiology is restored.

Reference.

VAUGHAN, G.L., and COOK J.S. (1972) : Regeneration of cation-transport capacity in HeLa cell membranes after specific blockade by ouabain. *Proc. Nat. Acad. Sci. U.S.A.*, 69, 2627-2631.

WOLPERT : It's a brave man who stands on this stage in the afternoon and wishes to introduce new systems and I am that brave man. We've heard quite a lot about differences between cells today and we've also heard something about gradients. What I want to very briefly talk about is how these differences between cells can arise and also something about how one might set up gradients. After all, as an embryologist I am primarily concerned with how you go from genetic information on the one hand to something like the hand on the other. It's the spatial organization that I want to talk about. Let me just make the point very clear because it can be a little confusing. Think about your arm, and your leg. You can regard them as comprising only two main cell types during early development, muscle and cartilage. From a molecular differentiation point of view, the process of becoming a muscle or cartilage cell in your arm and your leg is the spatial organization as to where you make muscle and to where you make cartilage. That is the pattern problem : how do you specify in space molecular differentiation ? The one class of mechanisms that I want to talk about uses gradients, or

what I would call positional information. I must first tell you that we're in a very primitive stage in this field. We know much less than anything else that's been talked about at this meeting so far.

The postulate is the following : one quite large class of mechanisms for giving spatial patterns is a two-step process : you give the cells what I call positional information, they have their positions specified with respect to certain boundaries : the second step is that they interpret this, that is they look up on the genome what to become (*Current Topics Devel. Biol.*, **6**. 183 (1971)). In other words, one would say for the arm and for the leg, that the cells would be given positional information with respect to certain boundaries : they would then look up, as it were, in the genome and decide whether to become muscle or cartilage. Thus pattern formation lies mainly in the response of the cells. There are several interesting features about this model. One is that the mechanism for providing positional information may be universal, it may be the same in all systems that make use of it. Another is that in all the situations that we know about, the distances over which a coordinate system is set up, are small. 1 mm or 50 cells long. The times on the other hand are relatively long, of the order of hours ; never of the order of minutes. It also begins to seem that, from looking at the systems studied, there are three parameters which are used in different ways. There is a stable cell parameter which I call the positional value ; there is some sort of communication between the cells which I call the positional signal (it may be a diffusible substance), and cell division. These seem, at present, to be the trinity on which various positional information systems are built up. I'm going to give you very briefly three examples.

The first system I'm going to deal with is in fact the arm, the chick wing (*Nature*, **244**. 493 (1973)). This seems to make use of a positional value and cell division without any signalling between the cells. The chick wing develops as a small outgrowth from the flank, and the skeletal elements are laid down in a proximo-distal sequence. We are concerned with this sequence of cartilaginous elements. The model that I want to suggest to you — partly based on our work — but largely based on the work of John SAUNDERS from Albany — is the following. Near the tip of the outgrowing limb is a special region which is specified by the epidermal covering. This region is called the progress zone. The way the cells get their positional information, along the limb, is essentially by a mechanism involving a clock. The rule is very simple. The longer you are in the progress zone, the lower your positional value. Since the width of the progress zone is constant and the cells are all dividing, they simply stop the clock when they come out of the progress zone. This will of course provide a gradient of cells as they leave the zone. Let me give you two pieces of evidence for this. The first is from the work of John SAUNDERS. If you remove the special ectodermal covering, you stop the clock. If this is done at an early stage, you lose distal parts. The second class of experiments, the ones we've done ourselves, is that if you do a transplantation, and swap young and old progress zones, the cells from these regions behave autonomously and as if they had not been moved. All they,re doing is measuring time. What you get in this situation is that a young progress zone transplanted to the tip of an older bud gives a second set of elements. We get a limb which goes humerus, radius and ulna, a bit of wrist, humerus, radius and ulna, hand. There is no signalling that we can detect whatsoever from the stump to the distal regions in the developing chick limb. You can see, incidentally, that the polarity, the vector in this case is time.

Let me go now to another system, hydra. All of you with your good classical education know that if you cut the head off hydra, it grows another one. It's a very nice system for looking at regulation. You can chop it about as you will, and it always makes anothers little one. In this case, cell division plays almost no role whatsoever and what

we seem to be dealing with here is positional value and a positional signal. The current model is based on cutting and grafting experiments. Hydra is very good for doing grafting experiments since you can change pieces from one place to another very easily and have a look whether it forms a new head in that region or not. The model suggests that there are two gradients : one is the positional value (P), which is more or less stable and one is the positional signal (S) which is possibly a diffusible substance made at one end by a source, destroyed at another end by a sink. The rule for making a new head, that is the rule for establishing the boundary region of this coordinate system is if S falls a critical distance below P. When S falls a critical amount below P, a new head end formation is initiated. If we cut off the head of this animal, we've cut away the source of S. I want to give some evidence that is at least consistent with diffusion and shows that time for signalling by S is long (*Nature*, **239**, 101, (1972)). The experiment is the following : if we can keep S up we will never form a new head. In a way you can regard S as an inhibitor of head formation because if S is not allowed to fall below P, no new head will form. The experiment is very simple. You take the head end of one animal and you put it at the proximal end of another animal. Can this grafted head inhibit the formation of a new head here when we cut off the host head ? As WILBY and WEBSTER showed, you can in fact do this. What we have done is taken it one step further We've asked, how do the times vary with distance ? How long must we leave the grafted head on here before chopping off this one so that a new head will not form here ? We ask how that varies with the distance. Two important results come out of this. First, that it's very sensitive to distance. Secondly, the times are long. It's not like the slime mould at all. When the distance is about 45 cells, you have got to graft the head on 8 hours before you take off the host to get inhibition. When you make the distance about 20 cells you can graft the head on 6 hours after you've already taken the host's off. So it is very sensitive to distance, the times become very long with increasing distance. If you do a plausible computer simulation what you can show is that it's in the right ballpark for diffusion. It doesn't show it is diffusion, but you can make a plausible case for diffusion.

Finally I want to mention a system where all three parameters of these seem to be involved. That is in the insect segment. The first is the work of Peter LAWRENCE and Francis CRICK in Cambridge (*J. Cell Sci.*, **11**, 815 (1972)). What they've shown is that you can use gradients — once again, you have to use a double gradient — to explain polarity in the insect epidermis. The insect epidermis is very beautiful because you have local markers of polarity. You've got bristles that point from the front end to the back end. The argument is that polarity is determined by the sign of the slopes of the gradient. The bristles will always point down the gradient. What they have is data showing that if you graft from a low point into a high level then you would expect to get a local reversal of the sign of the gradient and what they get is that the bristles now point locally in the opposite direction. I do not have time to discuss their more detailed experiments here.

What I'm really trying to say is that we may — if we're lucky — have one class of mechanism which is common to a large variety of systems. It could provide the cells with positional infomation using only three parameters which in different systems may all be the same, even at the molecular level — that's the provocation and the hypothesis — and hopefully this might give us some insight into pattern formation.

EBERT : Thank you... questions ?

SEGEL : Diffusion is supposed to take 4 times as long to go twice as far. Is it possible to get anything from your delay experiments on that point ?

WOLPERT : One of the main points of our analysis is that one cannot use such a simple argument. The reason is, you.re changing the geometry in different situations. In one situation you have two sources at the same time and in another situation you have only one source. The answer is that this is consistent with diffusion if you stimulate the system on the computer.

EBERT : I wonder, Dr. SEGEL, if you'd tackle a question for me. How did you decide to use the predator prey model in analyzing cell death in morphogenesis ? It seems foreign to me, from what I know of the process. Perhaps for the nonbiologists one might first of all indicate the significance of this point. WOLPERT has been talking about the development of the limb. In the development of the limb in most verterbrate organisms, the sculpturing or the shaping of the limb results not only from synthetic steps but from the death of selected cells. For example the fingers are shaped by the precise loss in space and time of the cells which normally lie between them. — It starts out as a solid mass and then cells progressively die. How did you arrive at the predator prey concept ?

SEGEL : Nothing very fancy, I'm afraid. I know from the papers of SAUNDERS of this phenomenon and it just sort of made a little click... to eat something or to kill it is more or less the same. Formally there's a good deal of similarity between the two things. That's all.

EBERT : But what puzzles me is that this does not appear to be murder — this is " suicide ". The cell appears to kill itself.

SEGEL : Right, but I suggested in an entirely tentative way that a cell could secrete a chemical which affected it adversely. This is a sort of suicide.

EBERT : I don't think that there's any evidence that anything is secreted by the cell...

SEGEL : I only made a tentative suggestion. One knows that there is patterned cell death. I tried to envision a way to del * a pattern.

EBERT : Any other questions for Dr. SEGEL specifically ?

Dr. COHEN, are you still of a mind to give us five minutes or so — and I say five minutes or so because I do want to save some time for Prof. MORGENSTERN.

M.H. COHEN : Thank you — just what does " or so " mean ?

EBERT : Six.

M.H. COHEN : In the Tuesday and Thursday morning program, there was one talk each which concerned local character and one global questions. There has not been very much said in fact about the connection between the local processes discussed in considerable detail by Dr. EDELMAN and Dr. MOSCONA, and the questions of global control or global organization that was discussed by myself and Dr. SEGEL. Actually Louis WOLPERT mentioned the word which contains the connection, which is *interpretation*. At the level of biological organization from which I viewed development and which is implicit in

* *Note added after the discussion.* In private conversation Dr. EBERT convinced me that my " self-secreting poison " model was not in accord with SAUNDERS chick experiments. I therefore deleted the relevant paragraph from the written version of my paper. L. SEGEL.

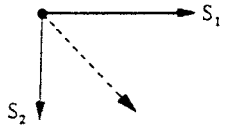
many of the discussions given one is concerned with cellular events and their control at the multicellular level. At that level, development takes place by approximately 6 unitary processes. The particular process that I was concerned with was movement. The process that Dr. MOSCONA was concerned with was a particular kind of adhesive connection between cells, contact formation between cells which ultimately led to the morphogenesis. It is quite possible that the same dynamical processes that are involved in development are also involved in its control, and these change on essentially the same time scale. In that case there would be no real point in trying to separate out of the dynamical system a set of control variables and describe how these evolve and what the connection is between the developmental variables and the control variables. But we have many examples where we can isolate and identify the control variables and establish that they change on a time scale which is something on an order of magnitude shorter than the time scale of the developmental variables. For example, the periods between the major stages in the life cycle of the slime molds are of the order of hours, and the time it takes for the actual propagation of a signal across a field, or territory, is of order of minutes. An other example is the migration of cells to form the heart primordia and the migration of the primordia to the midline, which can be observed in suitable time-lapse films of chick development. This again suggests the possibility of an external control system which is distinct from the local processes directly involved in development, movement and contact formation. There can thus be two quite separate aspects of development : the local cellular processes involved in development, in which cell behavior is essentially autonomous in response to an external signal, and the global system which provides the signal. The connection between these is the concept of interpretation of Prof. WOLPERT's global signal-local response. In the cellular slime molds the local response was a movement: the global control ssytem was signal propagation and the link between them, the interpretation, so to speak, was through chemotaxis.

BENNETT : Many years ago I was a pupil of Professor Arturo ROSENBLUTH, a very interesting Mexican physiologist who collaborated some with Norbert WIENER (1). I remember very well a set of papers, in one of which they treated systems of which cardiac muscle was an example. The essential features of the systems were as follows. They conceived of a random distribution of points. Each of these points was capable of receiving a signal. After receiving the signal, and after transmitting it, the point would have a period of incapability when it couldn't broadcast again. This would correspond to an immunie or a refractory period. They specified further that the signal would be transmitted at a finite velocity from each point in all directions. They then described the behavior of the passage of this signal in a system made up of many points capable of this kind of behavior. Thus an individual who receives the virus of an epidemic disease behaves as such a point. He becomes capable of transmitting the virus and then goes through an immune period. The signal, which is the virus, is transmitted in random directions. After a period of immunity the person recovers the capability of being reinfected. Such systems are formally very much like the system that COHEN and ROBERTSON have been studying. There principles are also applicable to the conduction of the impulse in the heart.

EBERT : In opening this mornig's session, the chairman observed that the biologists were instructed to speak to the physicists and the physicists to the biologists. Prof. MORGENSTERN is now going to speak to both of us. Whether he's going to say something we can *all* understand or something that *none* of us can understand, I don't know, but he promises to treat us equally.

MORGENSTERN : I am, as you know, a complete outsider in this conference as far as the topics are concerned. Being a social scientist, I must first of all say that I am enormously impressed by the depth and extent of your konwledge and procedures and by the methods used. I envy you the ability to make experiments which are very difficult to make in the social sicences. Having said this I will add even that social science theory is a million miles away from the theories of physics or chemistry and probably also from biology, although on the other hand I must say that I've tried to find out what " theoretical biology " is and I haven't quite got many explicit theorems presented to me (that on the side). The first thing to realize is that the social sciences are very differently structured than any of the physical sciences and even the biological sciences. One of the reasons, of course, is that one has to deal with conscious agents, human beings who operate. They may have antagonisms, or they may wish to cooperate ; they have different degrees of information, they have aims and all this is very hard to interpret in physical terms. Nevertheless economic theory, which is of all the social sciences probably the most advanced, has modeled itself after physics. This is understandable, because physics is such a fabulous science. It has so much to show. Expecially mechanics is inseparable from the development of calculus and the whole of that branch of mathematics. So, at the moment as one wishes to mathematize economic phenomena one will quite naturally look for some discipline which has been successful in using and developing mathematical methods. This has dominated economics and also to some extent sociology over the last generations.

But it is interesting that it was a very eminent physicist who has, as far as I know, first pointed out what the differences are and let me give you the illustration he gave. It was none less than PLANCK, who said that if you have an object and you have two forces working as in this figure :



then it's pretty obvious that the resultant of the two forces will be what happens to that object. Then he said, perhaps you have a dog here instead of an inaminate object. You place one sausage here at S_1 and another sausage there at S_2. It is most unlikely that the dog will run according to the parallelogram of forces (laughter)... That's the difference ! But the economists have not observed this. I have looked at PLANCK's writings, but I can't find the reference to this observation. I hope to discover it — if anyone can contribute the reference to me some time I would be most grateful, because I'm writing a history of game theory and I would like to put this in, because I think the remark was made before 1928 when von NEUMANN wrote his important paper on game theory *. It would be nice to point this out.

Let me now say that the physical analogies lead to the fact that one wishes to obtain a minimum of cost or a maximum of revenue or a social maximum and so on. One realizes, of course, that if one does this one might have to obtain maxima which are under constraints. But the point is that whether this works at all : because, in order to have a maximum which you can find and set, you've got to control all variables. The point, of course is, that you do *not*, in political and economic affairs, control all the variables. One might argue that you don't even control nature. But nature, we assume, is indifferent to us — perhaps it is questionable, whether nature is not hostile, but let's us

* Later I have discovered that the observation is due to NERNST (added in proof).

assume it's indifferent. If I'm a farmer, an isolated farmer, I can maximize what I plant because I can treat nature statistically; it might rain more one year than the other but I can average this out by the proper choice of cultivation. But if I am in contact with other people our interests will in part be totally opposite and this means we will be in opposition, but in part I will perhaps want to cooperate. The total opposition, of course, is clear if you fight a duel and you will immediately see what difficulties there are in decision making: two of us have gotten into this unfortunate position, we stand back to back; each one has a pistol with one shot and we walk away from each other and then are told to turn around and to walk towards each other. The nearer I come the more likely I will be able to hit the other fellow, but then the more likely he will hit me, and if I fire early and fail, then I'm surely dead and vice versa. So what is the right decision? That is a problem which has absolutely no parallel in physics, or chemistry and, as far as I can see, perhaps not even in biology.

In situations similar to duels it is a question of choosing the right strategy and to develop a decision rule. The decision rules can be very difficult to discover. One can do it in two ways. If it is a very complex situation in which you find yourself — a game of chess or something similar — you could describe it in the " extensive " form, that is, step by step, move by move, which together make up the strategy. Or, as one can prove — the optional procedure can also be described in a " normalized " form where all the conceivable consecutive steps are bound together into one strategy. To make it very simple I will give an illustration of the following kind. Because of the time pressing, I will go right away to a more complicated situation in order to show the difficulties and at the same time to bring out what actually happens. Suppose the police capture two people. They are suspected — one has found them violating some traffic rules; they were taken but at the same time there is a suspicion that they have committed a major crime. They are immediately separated and held incommunicado. Now they are told that they are suspected of a grave crime. They are asked to confess to the crime. If they both confess they will be punished, but if one does not confess and the other confesses, then he who does not confess will be most severely punished but the one who confesses will receive a lesser punishment. If both confess to the crime they will, for example, each get 5 years; they are treated equally. If one confesses and the other does not confess, then the first one gets only one year and the other gets ten years in jail. But if both do *not* confess, they are being punished for whatever the crime is that they've been discovered having actually committed, such as carrying weapons. What is the best strategy now? You see the elements that enter — I will not analyze them in detail because there's no time for it — but it is clear that if the two men have absolute confidence in each other, neither will confess; but it is a very risky thing to do, because the other might after all confess. The difference between ten years and one year is substantial. This is the famous game of prisoners' dilemma. The obvious thing is that they would be both wise to confess, because each alternative is somehow worse.

This is again a situation you cannot analyze by any device coming from physical science. Total antagonism is, for example, shown in a highly significant case as follows: You play a game which is called matching pennies. There are two players, each one has a penny and they put it down and if they show matching heads or tails, then he who plays for matching gets one unit and he who plays for not matching loses the unit. So the first player has his strategy to choose: either he has to show heads or tails, and the other player has the same strategic possibility and they are all true. One cannot imagine a simpler situation than this one. And yet it brings out something fundamental. The numbers in this table mean that the payments go from the second player to the first — merely a convention.

Player 2

Player 1		H	T
	H	1	− 1
	T	− 1	1

In case of matching (on either Heads or Tails), the second player pays one unit to the other — otherwise it is the other way around. The game has to be played in the open. The one who starts first is licked because the other will do exactly the opposite. The antagonism between the two is total, just as in the duel.

Now it is clear what the proper policy must be: You will not chose the strategy yourself, rather you will invent a chance device which will choose the strategy for you, the reason being that you must under all circumstances prevent giving away information, and the best way not to give away information is not to have any. The chance device in this case is the coin itself. You don't have the information you want to hide, because you don't know which side the coin by chance will turn up.

If each tosses the coin — and both do it simultaneously — the naive observer will interpret this game as a game of chance. That is wrong. It is *not* a game of chance. It is a game of strategy in which a chance device is introduced which will determine the outcome. But it must be the *right* chance device — which shall determine the outcome. I can immediately change the example to illustrate this remark. For example, if this were a matching on heads and there would be double payment if it matches, then it is obvious that the 50-50 chance device represented by the coin is no longer correct. Instead, one has to calculate on the whole basis of game theory, which I cannot possibly describe here, the proper mixture of strategies with which each one must play these strategies. So you see the question as to what is the right strategy is far from trivial. That an optimal strategy always exists for any zero sum game is based on the famous minimax theorem by John von NEUMANN which was originally proved in 1928 when he was a very young man. It was a very deep and complicated proof using fixed point theorems and other methods which are not encountered in physics. Thus, there is no similarity with physics, and the differential calculus does not play any role whatsoever in the whole matter here. Although widely used so far, attempts to bring mathematics to bear economic and socio-logical problems the success has been limited.

Let me go onto the next item. I will make it as short as I can. I want to say a word about the question of the stability of a solution of an n-person situation where more than two people are involved. Whenever one makes a transition from one player to two or more, significant changes occur. One player, all alone, plays solitaire — that's of no interest whatsoever. If you go over to two, you may have the complete antagonism just described. But you may *not* have complete antagonism, and then the game is not zero — sum, because — different from matching pennies — both may win or lose. For example, if I want to buy something from you, there is a certain antagonism because you want to sell at as high a price as possible and I want to buy at the lowest price possible, that is the opposition — but nevertheless we have a joint interest also, namely we want to get a transaction actually going. So there is a joint interest combined with an opopsition. That is a very interesting and socially characteristic phenomenon. Clearly, other optional strategies must be determined and that can be done using the fundamental minimax theorem mentioned earlier.

Let us now look at a situation where three people are involved. Here, something entirely different begins to happen. If you go to four and five and so on, the matter

becomes exceedingly difficult, although a general theory can be and has been set up. Let me write down...

		Players		
		1	2	3
α	(1,2)	1/2	1/2	− 1
β	(1,3)	1/2	− 1	1/2
	(2,3)	− 1	1/2	1/2

Here you have three players and the game is that two of them, if they combine, extract one unit from the one who is excluded. Very simple. Now there are obviously only the following possibilities — one and two combine, one and three can also combine or two and three combine. But these are the only possibilities. The losing player has to pay one unit, that is the rule which is unbreakable. My assertion is that the solution is that this unit will be distributed among the others in this manner: 1/2, 1/2 although obviously there exists an infinity of ways in which the one unit can be distributed. So why would that then be the solution? Let us call the three distributions, or imputations, alpha, beta and gamma. These imputations are the arrangements made in order to settle the game. Now consider another one, another distribution, let us say that one of the players has a privilege. The rules may say that if he is in the winning coalition, he must get an amount epsilon, which is greater than 0 and smaller than 1. Then this distribution, if he is present, would be one half plus epsilon, the other can only get 1/2 minus epsilon and — 1 i.e.: $1/2 + \varepsilon$, $1/2 - \varepsilon$, -1. Call this imputation $\hat{\varepsilon}$. Let us now look at the entirety of this very simple scheme of Fig. 2. We ask ourselves why do such distributions have special interest? They are acceptable because they have a certain stability, but the stability does not lie in its own single activation, in its own single characteristic. Neither α, nor β, or γ is " stable " taken by itself. Stability lies only in the relation each one of them has to the others. What are these relations? Let's look at the imputation $\hat{\varepsilon}$. Here we find that the first one who is priviledged is better off in $\hat{\varepsilon}$ than in β because he gets epsilon more besides 1/2. The second one is better off in $\hat{\varepsilon}$ because though he gets not 1/2, but he gets $1/2 - \varepsilon$ which is obviously better than — 1 which he would get in β. So the third loses. We can say that delta, " dominates " beta. Two are individually better off. They would therefore prefer this arrangement. Yet each is driven to seek the best he can obtain. Therefore $\hat{\varepsilon}$ will have to be compared with α and γ as alternatives. β is ruled out because it is dominated by $\hat{\varepsilon}$. Our Table now shows us that in γ players 2 and 3 are individually better off than in $\hat{\varepsilon}$, hence γ dominates $\hat{\varepsilon}$ and $\hat{\varepsilon}$ annot be considered a stable arrangement. But now if you look at alpha, beta and gamma you will not find that any one of them dominates any other in the table. Therefore as an entirety, as a set, they constitute the " solution ", although each one of them is threatened by some delta or êta or whatever you have. But each one which is threatened, as for example beta is threatened by delta, is in turn protected by the fact that there exists another arrangement *belonging* to the solution which kills or threatens or removes from consideration the one which is outside the solution.

I have said that the first player was privileged in the rules of the game discussed earlier, in the sense that he had to get the extra amount ε if he belonged to a coalition. What can he do? If he insists on his privilege he will never be considerer. So he has to offer the epsilon as a side payment in order to be accepted for possible consideration. It is clear that this is totally different from anything considered in physics or in other natural

sciences. I might even point out, incidentally, that as simple as this solution scheme looks, the mathematical structure has very peculiar properties. It is not even a partially ordered set. If you have such orderings, then one is confronted with different matters than those to which ordinary optimum and maximum notions apply as commonly used. Therefore if one thinks, for example, that one can construct a " social optimum " in any naive, clear way, one is far away from seeing the underlying, fundamental difficulties and the mathematical and logical realities. This account gave perhaps a brief indication as to what is involved in this field. Of course, behind my sketch stands, as I've said, a very elaborate mathematical theory.

It is far from finished. It is being developed actively in many countries. The theory attempts to deal with those characteristics of social behavior which are common to all forms of organization, involving self-interest, states of information, cooperation, antagonisms, etc. It aims to discover what fairness means, it shows that symmetric arrangements may however work out a-symmetrically, how prejudices influence behavior and discrimination can become a force etc. I was induced to make these remarks because it seems to me important that one realize that notions of stability of systems can differ widely depending on the inner structure of the sciences to which this concept is applied. And the social sciences differ significantly from those devoted to natural phenomena.